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Award Number: DAMD17-03-1-0522

TITLE: Constitutive Activation of NF- κ B in Prostate Carcinoma Cells Through a Positive Feedback Loop: Implication of Inducible IKK-related Kinase (IKKi)

PRINCIPAL INVESTIGATOR: Irina V. Budunova, M.D., Ph.D.

CONTRACTING ORGANIZATION: Northwestern University
Evanston, Illinois 60208

REPORT DATE: August 2004

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PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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The overall goal of this project is to understand the role of inducible IKK-related kinase IKKi in constitutive activation of anti-apoptotic transcription factor NF- κ B prostate carcinoma (PC) cells. We found that IKKi is highly expressed in PC cell lines with constitutively activated NF- κ B. We also performed immunostaining of more than 60 PC and BPH samples using four different antibodies against IKKi. Immunostaining revealed that IKKi was highly expressed in prostate glands, but not in prostate stroma. IKKi signal had both cytoplasm and nuclear localization. Overall intensity of IKKi staining was similar in BPH and in PCs, however, the nuclear expression was higher in PCs. Treatment of PC cells with different NF- κ B inducers such as IL-1 alpha, TNF-alpha, and TPA resulted in a rapid induction of IKKi. Consistent with this, down-regulation of NF- κ B activity by proteasome inhibitor MG132 and IKK inhibitor PS1145 attenuated induction of IKKi expression by NF- κ B inducers. Transient transfection of different PC cell lines with IKKi w.t. resulted in activation of κ B.Luciferase reporter, whereas IKKi dominant negative mutant suppressed basal NF- κ B activity in PC cells. These data provide experimental evidence that IKKi could be involved in the regulation of NF- κ B activity in PC cells through a positive feedback loop.

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Introduction

The overall goal of this project is to understand the role of inducible IKK-related kinase IKKi in constitutive activation of anti-apoptotic transcription factor NF- κ B prostate carcinoma (PC) cells. During FY01 we confirmed that IKKi, whose expression is currently linked mostly to the cells from lymphoid tissues (Shimada et al., 1999), is highly expressed in PC cell lines with constitutively activated NF- κ B. We also performed immunostaining of more than 60 formalin-fixed paraffin-embedded samples (including tissue microarrays and individual sections) of BPH, and PCs using four different antibodies against IKKi. The analysis of IKKi staining in prostate tissue samples indicated that IKKi was highly expressed in prostate glands, but not in prostate stroma. IKKi signal had both cytoplasm and nuclear localization. Overall intensity of IKKi staining was similar in BPH and in PCs with Gleason scores 6-9, however, the nuclear expression was higher in PCs. Treatment of PC cells with different NF- κ B inducers such as IL-1 α , TNF- α , and TPA resulted in a rapid induction of IKKi. Consistent with this, down-regulation of NF- κ B activity by proteasome inhibitor MG132 and IKK inhibitor PS1145 attenuated induction of IKKi expression by NF- κ B inducers. Transient transfection of different PC cell lines with IKKi w.t. resulted in activation of κ B.Luciferase reporter, whereas IKKi dominant negative (d.n.) mutant K38A suppressed basal NF- κ B activity in PC cells. These data provide experimental evidence that IKKi could be involved in the regulation of NF- κ B activity in PC cells through a positive feedback loop. In addition, I would like to emphasize that we were able to successfully work in frames of the proposed project despite the fact that during the current funding period my laboratory has moved from AMC Cancer Center in Denver to Northwestern University in Chicago. During first several months of transition period I had to rebuild my laboratory, transfer awards to NU, hire new postdoctoral fellows and technicians; prepare new IRB and IACUC protocols. I have accomplished all these goals successfully. In addition, I became a member of NU Cancer Center, the member of NU prostate SPORE program, and a member/mentor of NU integrated graduate program. My laboratory also participates in summer student training program, and this year one of the summer students is involved in the studies focused on the role of NF- κ B in prostate tumorigenesis funded by DOD. The results of our studies have been presented at national meetings, one manuscript is under preparation. The following describes progress made in this year.

Body

During current year we specifically focused on the experiments pertinent to the tasks 1 and 2.

Task 1. To determine the role of IKKi in NF- κ B activation in prostate we first confirmed that IKKi is highly inducible in PC cell lines with either high or low constitutive level of IKKi expression. We found that treatment of PC cells (LNCaP cells that do not express IKKi constitutively, and DU145 and PC3 that express IKKi) with NF- κ B inducers such as IL-1 α and TNF- α resulted in a rapid induction of IKKi. The increase in expression was obvious in 2-4 hr depending on cell line, and lasted for 24 hr. TPA appeared to be less active as IKKi inducer. Sensitivity to IKKi induction correlated well with sensitivity of specific cell line to NF- κ B induction by different inducers. Consistent with this, down-regulation of NF- κ B activity by proteasome inhibitor MG132 and IKK inhibitor PS1145

attenuated induction of IKKi expression by NF- κ B inducers. Chemical NF- κ B inhibitors also inhibited the constitutive IKKi expression especially in PC3 cells. Surprisingly the treatment of DU145 and PC3 cells with NF- κ B inhibitor, synthetic glucocorticoid fluocinolone acetonide did not affect IKKi expression. Importantly, the conditioned medium from PC3 cells induced IKKi expression in LNCaP cells suggesting that IKKi expression maybe regulated through autocrine loop/loops in PC cells. The role of paracrine and autocrine loops in NF- κ B activations during tumor development in different tissues is strongly considered (Greten and Karin, 2004).

To assess the effect of w.t. IKKi and kinase inactive IKKi mutant, K38A (Peters et al., 2000) on NF- κ B activity in PC cells we performed transient transfection of two different PC cell lines LNCaP and PC3. In these experiments we used two sets of effector constructs: constitutively active IKKi constructs with w.t. mutant IKKi under CMV promoter (kindly provided by Dr. Maniatis, Harvard Medical School, Cambridge, MA) and inducible IKKi constructs under minimal promoter with kappaB-binding site (kindly provided by Dr. Mercurio, Signal Pharmaceuticals, Inc., San Diego, CA). The last set of constructs is NF- κ B -inducible and mimics the "natural" expression regulation pattern of IKKi in cells. We used different kappaB reporters for transfection experiments with kappaB sites from selectin gene promoter (selectin. κ B.Luc), from the promoter of interferon-gamma (INF. κ B.Luc) and x5. κ B.Luc reporter with five conventional kappaB binding sites. The most reliable results have been obtained with INF.Luc. and x5. κ B.Luc reporters. Analysis of transient transfection experiments revealed that overexpression of IKKi w.t. resulted in activation of κ B.Luc reporters, whereas IKKi dominant negative mutant K38A suppressed basal NF- κ B activity in PC cells.

We also performed stable transfection of PC cells with high constitutive IKKi expression with CMV.IKKi d.n. construct. The blockage of IKKi function by stable transfection resulted in the decrease of constitutive expression of endogenous kappaB-responsive genes I κ B- α and IL6. Overall, these data provide the experimental evidence that IKKi could be involved in the regulation of NF- κ B activity in PC cells through a positive feedback loop.

Task 2.

As proposed, we built the collection of formalin-fixed and frozen prostate tissue samples using NCI Cooperative Human Tissue Network (CHTN) and also resources of NU prostate SPORE core. We performed immunostaining of more than 60 formalin-fixed paraffin-embedded samples (including tissue microarrays and individual sections) of BPH, high grade PIN, and PCs using multiple antibodies against IKKi (four different Abs from Imgenix, Santa Cruz, Active Motif, Pro-Sci). The analysis of IKKi staining in prostate tissue samples indicated that IKKi was highly expressed in prostate glands, but not in prostate stroma. IKKi signal had both cytoplasm and nuclear localization. Overall intensity of IKKi staining was similar in BPH and in PCs with Gleason scores 6-9, however, the nuclear expression was higher in PCs. In addition, we analyzed expression of NF- κ B (p65- two different Abs from Abcam and Santa Cruz; and p50 - Santa Cruz), one of the possible targets of IKKi - IKK β (Imgenix). We found that there was relatively modest increase of number of p65-positive nuclei in low grade and advanced PCs in comparison to BPH. There were no significant changes in the expression of p50 and IKK β in PC in comparison to BPH. We have successfully isolated total RNA from

frozen PCs and BPH samples, and started to perform Northern blot analysis of the expression of kappaB-dependent genes.

Next year we will continue to evaluate the expression of IKK target proteins in prostate tissues using Western and Northern blotting, and RT-PCR. We will use combination of co-immunoprecipitation and protein chromatography to reveal the interaction of IKKi and its targets in PC cells.

Key Research Accomplishments

- ❖ We found that IKKi is expressed in glandular component of prostate samples. Nuclear IKKi expression was increased in human prostate carcinomas in comparison to BPH samples.
- ❖ IKKi is highly inducible in PC cell lines. Treatment of PC cells with NF- κ B inducers such as IL-1 α and TNF- α resulted in a rapid induction of IKKi. TPA appeared to be less active as IKKi inducer.
- ❖ Down-regulation of NF- κ B activity by proteasome inhibitor MG132 and IKK inhibitor PS1145 attenuated induction of IKKi expression by NF- κ B inducers. Both inhibitors also decreased the constitutive IKKi expression in PC3 cells.
- ❖ Transient transfection of different PC cell lines with w.t. IKKi resulted in activation of κ B.Luciferase reporter, whereas IKKi dominant negative (d.n.) mutant K38A suppressed basal NF- κ B activity in those cells.
- ❖ The blockage of IKKi function by stable transfection of PC3 cells with IKKi d.n. mutant resulted in the decrease of constitutive expression of endogenous κ B-responsive genes I κ B- α and IL6.
- ❖ These data provide experimental evidence that IKKi could be involved in the regulation of NF- κ B activity in PC cells through a positive feedback loop.

Reportable outcomes

Manuscripts

Yemelyanov A., Yao Y., and Budunova I. Possible role of IKKi in the constitutive activation of NF- κ B in prostate carcinoma cells. Under preparation.

Abstracts presented at national meetings

1. Yemelyanov A., Yao Y., and Budunova I. Possible role of IKKi in the constitutive activation of NF- κ B in prostate carcinoma cells. Keystone Symposium: NF- κ B: biology and pathology. January 11-16, 2004, Snowbird, Utah, p. 101.
2. Budunova I., Yemelyanov A., Gasparian A., Dang L., Pierce J. Effect of IKK-beta specific inhibitor PS1145 on NF-kappaB activity and apoptosis in prostate carcinoma cell lines. . Proceedings of AACR 45, 2004 (abstract # 4572).
3. Yemelyanov, A., Yao, Y.J., and Budunova, I. IKKi is a component of the positive feedback loop involved in the constitutive activation of NF- κ B in prostate carcinoma cells. Proceedings of AACR 44: 852, 2003.
4. Gasparian, A. V., Yao, Y. J., Slaga T.J. and Budunova, I. V. High sensitivity of prostate carcinoma cell lines to NF- κ B induction. Proceedings of AACR, 44: 1451, 2003.

Seminar presentations by PI

1. Constitutive activation of NF- κ B in prostate carcinoma cells: possible role of feedback loop involving IKKi. Department of Urology seminar program, Feinberg School of Medicine, Northwestern University, Chicago, September, 2003.

Conclusions

Our data provide experimental evidence that IKKi could be involved in the regulation of NF- κ B activity in PC cells through a positive feedback loop.

We found that IKKi is expressed in glandular component of PC and BPH prostate samples. Immunostaining of prostate tissues revealed that nuclear IKKi expression was increased in human prostate carcinomas in comparison to BPH samples.

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1. Peters RT, Liao SM, Maniatis T. IKKepsilon is part of a novel PMA-inducible IkappaB kinase complex. *Mol Cell.* 5: 513-522, 2000.
2. Shimada, T. Kawai, T., Kiyoshi, T., Matsumoto M., Inoue, J., Tatsumi, Y., Kanamura, A., and Akira S. IKK-i, a novel lipopolysaccharide-inducible kinase that is related to I κ B kinases. *International Immunology.* 11: 1357-1362, 1999.
3. Greten FR, Karin M. The IKK/NF-kappaB activation pathway-a target for prevention and treatment of cancer. *Cancer Lett.* 206(2):193-199, 2004.

Appendices

1. Yemelyanov A., Yao Y, and Budunova I. Possible role of IKKi in the constitutive activation of NF- κ B in prostate carcinoma cells. Keystone Symposium: NF- κ B: biology and pathology. January 11-16, 2004, Snowbird, Utah, p. 101.
2. Budunova I. , Yemelyanov A., Gasparian A., Dang L., Pierce J. Effect of IKK-beta specific inhibitor PS1145 on NF-kappaB activity and apoptosis in prostate carcinoma cell lines. . Proceedings of AACR 45, 2004 (abstract # 4572).
3. Yemelyanov, A., Yao, Y.J, and Budunova, I. IKKi is a component of the positive feedback loop involved in the constitutive activation of NF- κ B in prostate carcinoma cells. Proceedings of AACR 44: 852, 2003.
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Importance of NF- κ B in neointimal formation after balloon injury in porcine coronary artery

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Division of Clinical Gene Therapy, Osaka University Graduated School of Medicine,
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Percutaneous transluminal coronary angioplasty (PTCA) is a modern treatment regime of coronary diseases. However, restenosis after PTCA is a serious problem. Although mechanisms of restenosis remain unclear, the proliferation and migration of vascular smooth muscle cells (VSMC), and local inflammation are generally considered to play an important role in the progression of atherosclerotic plaque and subsequent restenosis of coronary arteries. The transcription factor, nuclear factor-kappa B (NF- κ B), plays a pivotal role in the transactivation of cytokine and adhesion molecule genes. We hypothesized that inhibition of NF- κ B activation may lead to prevent restenosis after balloon injury. To prove this hypothesis in this study we examined the effect of cerivastatin, one of the HMG-CoA reductase inhibitors, that is known to inhibit NF- κ B activity and NF- κ B decoy oligodeoxynucleotides (ODN) on neointimal formation after balloon injury in porcine. Vehicle (n=8) or cerivastatin (n=8) was orally administered at 1mg/kg/day from 7 days before lasting up to 4 weeks after balloon injury. We also transfected NF- κ B and scrambled decoy ODN into the balloon-injured artery using a hydrogel catheter. After 4 weeks, the histological staining demonstrated a significant inhibition of neointimal formation by cerivastatin and NF- κ B decoy ODN (p<0.01). In addition, the impaired response of endothelium to bradykinin in balloon-injured vessels was significantly improved by treatment with cerivastatin and also NF- κ B decoy ODN (p<0.05). In these analysis vehicle or scrambled decoy ODN had no effects. Overall, the present study indicates that inhibition of NF- κ B activity by cerivastatin or decoy ODN has a direct inhibitory effect on neointimal formation and improvement of endothelial dysfunction. These data suggest the importance of NF- κ B in the restenosis and also that NF- κ B decoy ODN approach could be an effective strategy for restenosis after coronary angioplasty.

1)Keita Yamasaki, 2)+81-6-6879-3401, 3)A4

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Stimulus-specific Induction of I κ B- ζ , a Novel Regulator of NF- κ B

Soh Yamazaki,¹ Tatsushi Muta,^{1, 2} and Koichiro Takeshige,¹ ¹Dpt. Mol. Cell. Biochem., Grad. Sch. Med. Sci., Kyushu Univ., Fukuoka 812-8582 and ²PRESTO, JST, Saitama 332-0012, Japan

We have recently identified a novel nuclear protein, I κ B- ζ . I κ B- ζ preferentially associates with the NF- κ B subunit p50 rather than p65, through which it regulates the activity of NF- κ B. I κ B- ζ is hardly detectable in resting cells such as macrophages and fibroblasts and is induced by various microbial components including lipopolysaccharide (LPS) and peptidoglycan, or by the inflammatory cytokine interleukin (IL)-1 β . Tumor necrosis factor (TNF)- α , however, does not elicit the induction of I κ B- ζ although it induces the activation of NF- κ B or p38 MAP kinase as LPS or IL-1 β does. Analyses on the promoter and studies using various inhibitors indicated that NF- κ B was essential for the induction of I κ B- ζ . Transcriptional activation, however, does not appear to account for the stimulus-specific induction, since the nuclear run-on assay indicated that LPS, TNF- α , or IL-1 β up-regulated the transcription of I κ B- ζ to the similar extent. Then, we established NIH/3T3 cell lines constitutively expressing the full-length I κ B- ζ mRNA under the control of chicken β -actin promoter, and evaluated the stability of the mRNA. The decay of I κ B- ζ mRNA was specifically delayed when the cells were stimulated with LPS or IL-1 β , but not with TNF- α . These results indicate that the specific induction of I κ B- ζ is regulated at the level of the stability of mRNA, which is up-regulated by LPS or IL-1 β . The specific induction of I κ B- ζ might be critically involved in the regulation of NF- κ B-mediated transcription.

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Possible role of IKKi in the constitutive activation of NF- κ B in prostate carcinoma cells

Alexander Yemelyanov, Ya Juan Yao, and Irina Budunova
Northwestern University, Chicago, IL 60611

Our recent data and data by others indicate that NF- κ B is constitutively activated in androgen-independent prostate carcinoma (PC) cells and prostate tumors, and that NF- κ B activation promotes PC cells' tumorigenicity, invasiveness and resistance to apoptosis. The important step in NF- κ B activation is the phosphorylation of I κ B inhibitor proteins by IKK kinases: IKK α , IKK β and IKK-related inducible kinase IKKi. IKKi is the only IKK whose activity is regulated by its expression. We found that IKK α and IKK β were uniformly expressed in primary prostate cells and PC cell lines. On contrast, IKKi was strongly expressed only in androgen-independent PC cells (PC3 and DU145) with high level of constitutively active NF- κ B but not in androgen-dependent PC cell lines (LNCaP and MDA PCa 2b) and primary prostate epithelial cells. Immunostaining also revealed that IKKi was expressed in human prostate carcinomas. Treatment of PC cells with NF- κ B inducers such as IL-1 α and TNF- α resulted in a rapid induction of IKKi. Consistent with this, down-regulation of NF- κ B activity by proteasome inhibitor MG132 attenuated induction of IKKi expression by NF- κ B inducers. Transient transfection of different PC cell lines with IKKi w.t. resulted in activation of κ B-Luciferase reporter, whereas IKKi dominant negative (d.n.) mutant K38A suppressed basal NF- κ B activity in PC cells. These data provide experimental evidence that IKKi could be involved in the regulation of NF- κ B activity in PC cells through a positive feedback loop. Supported by DOD Prostate Cancer Research Program DAMD 17-03-1-0522.

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Stable Transfection Of A Mutated I Kappa B Alpha Into HNSCCa27 Cells Leads To Differential Sensitivity To 5-Fuorouracil and Cis-Platinum

Ming Yu, Carter Van Waes, Tumor Biology Section, Head and Neck Surgery Branch, National Institute on Deafness and Other Communication Disorders, National Institute of Health, Bethesda, Maryland

Cal27 is an established cancer cell line derived from a Head and Neck squamous carcinoma (HNSCC). This tumor cell line is tumorigenic in nude mice and has been shown to be resistant to a variety of chemotherapeutic reagents, including Cis-platinum and 5-Fluorouracil (5-FU) which are used to treat HNSCC. We have found that this cell line exhibits a high level of NF- κ B DNA binding activity and moderate NF- κ B-Luciferase activity. Un-transfected Cal27 carries detectable levels of I kappa B alpha. One of the stably transfected clones showed one-fold increase of I kappa B alpha protein level and about 75% decrease of NF- κ B luciferase activity compared to un-transfected Cal27 cells. This decreased NF- κ B activity doesn't inhibit cell survival and proliferation in these transfected cells, but does sensitize these cells to 5-FU 10,000 fold. Only about 20% increase in inhibition response to Cis-Platinum compared to un-transfected cells. Similar, but stronger, inhibitory effects by 5-FU were also observed in a sub-population of a pool of the transfected cells with lower increased I kappa B alpha protein level. Our result suggest that NF- κ B activity may play a greater role in resistance to 5-FU than Cis-Platinum.

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4572 Effect of IKK-beta specific inhibitor PS1145 on NF-kappaB activity and apoptosis in prostate carcinoma cell lines.

Irina V. ■Budunova■, Alexander Yemelyanov, Alexander Gasparian, Lenny Dang, Jacqueline Pierce. *Northwestern University, Chicago, IL, National Cancer Research Center, Moscow, Russian Federation, Millenium Pharmaceuticals Inc., Cambridge, MA.*

Prostate cancer (PC) is the second leading cause of death among cancers in men. One of the contributing factors to high mortality rate from PC is the extreme resistance of malignant prostate cells to apoptosis induced by radio- and chemotherapy. Thus, the specific induction of apoptosis in PC cells could play a strategic role for PC treatment. One of the central mechanisms protecting cells from apoptotic death is mediated by NF-kappaB factors that control the expression of numerous anti-apoptotic genes. We and others showed previously that NF-kappaB transcription factor was constitutively active in PC cell lines and in human prostate tumors due to the up-regulated activity of IkappaB-kinases (IKK), mostly IKK-beta. In this work we investigated effect of a novel highly specific IKK-beta inhibitor PS1145 on constitutive and inducible NF-kappaB activity in human cell lines PC-3 and DU145 using Luciferase Assay with x5.kappaB-Luciferase reporter, EMSA, Northern blot analysis of expression of endogenous kappaB-responsive genes, Western blot analysis of IkappaBalpha phosphorylation, degradation and p65 nuclear translocation. Our studies revealed that PS1145 at the dose range 5-20 μ M efficiently inhibited both basal and induced by either TNF-alpha or LPS NF-kappaB activity in PC cells. PC3 and DU145 cells are known to be resistant to TNF-alpha-induced apoptosis partially due to the constitutively active NF-kappaB. We found that PS1145 significantly sensitized PC cell lines to TNF-alpha induced apoptosis. We observed the elevated PARP cleavage and caspase 3/7 activation when cells exposed to TNF-alpha were pretreated with PS1145. Currently we are evaluating the expression of kappaB-responsive genes as well as PC gene markers in prostate cells upon PS1145 treatment in vitro and in vivo. Supported by DOD prostate cancer research grants DAMD17-01-1-0015 and DAMD17-03-1-0522.

showed that the kinase activity of Fer (Fer dominant negative) was determinant for STAT3 phosphorylation in PC-3 cells but not for the formation of Fer/STAT3 signaling complexes. The significance of these *in vitro* findings was demonstrated by immunohistochemistry in human prostate specimens from patients with advanced prostate cancer where Fer and STAT3 were expressed in the nucleus of the same tumour cell populations. Altogether these findings show for the first time the implication of the Fer kinase in the modulation of prostate cancer cell growth by IL-6 and its major role in the activation of STAT3 up to the nucleus. We propose a novel role for Fer in maintaining elevated levels of pY-STAT3 for binding to the promoter region of IL-6 dependent target genes. Supported by the Cancer Research Society, Inc.

#4298 Role of p42/44 mitogen activated protein kinase signal transduction in PC3 cells, a line of hormone independent prostate cancer cells. Hari K. Koul, Lakshmi Chaturvedi, Mei Yi Haung, Akshay Bhandari, Mani Menon, Ira Wollner, George Divine, Raymond Demers, Anil Wall, and Roger J. Davis. *Henry Ford Health Sciences Center, Detroit, MI, Karmanos Cancer Institute, Detroit, MI, and Howard Hughes Medical Institute and Program in Molecular Medicine, Worcester, MA.*

Prostate cancer is the second leading cause of cancer related deaths in males. Over the last decade, significant progress has been made in various treatment options for localized prostate cancer including surgical refinements as well as establishment of Hormone and Radiation therapy. However, to date no satisfactory treatment options are available for hormone resistant prostate cancer. The present studies evaluated the role of p42/44 Mitogen activated Protein Kinases (p42/p44 MAP kinase) signal transduction pathway in growth, viability and clonogenic activity of hormone-independent prostate cancer cells. For these studies, log phase cultures of PC3 in DMEM/F12 medium supplemented with FCS (10%) and antibiotics were grown in multi-well plates in the presence or the absence of PD 098059, a specific inhibitor of MEK (the upstream activator of p42/p44 MAPK) for 2 to 9 days. At the end of pre-determined time points, the rate of DNA synthesis was evaluated by 3H-thymidine incorporation; Kinetics of cell growth were measured by measuring cell numbers at given intervals by crystal violet staining; MAP kinase activity was measured by Western blot analysis using specific antibodies; Low melt agarose cultures were used in clonogenic assays. PC3 cells achieved linear growth pattern up to 6 days in culture and the growth rate tapered thereafter. The maximum rate of DNA synthesis was observed on day 5, thereafter rate of DNA synthesis tapered. These data suggest that maximum number of cells were in S-phase of cell cycle on day 5. Exposure of the cells to PD 098059 (25 or 50 mM) had a modest effect on DNA synthesis in PC3 cells. Maximum effect of PD 098058 was observed on day 2 of exposure (~48% inhibition of the DNA synthesis with 50 mM, PD). The inhibition tapered by day 5 of exposure (to ~28% inhibition of the DNA synthesis with 50 mM, PD). PD 098059 decreased cell growth modestly (by 7-22%). Maximum inhibition of the cell growth was observed on day 5 of exposure (~22 % decrease in cell number with 50 mM, PD). The effects of PD 098059 on DNA synthesis and cell growth demonstrate that p42/p44 MAP kinase pathway plays only a minor role in the growth and viability of androgen-independent prostate cancer cells (PC3 cells). However, inhibition of ERK activity in PC3 cells had a profound effect (PD inhibited colony formation by over 80%) on clonogenic activity. These data suggest critical role for signal transduction via ERK pathway in synthesis and secretion of extracellular matrix components and thereby in tumor metastasis. Taken together these data demonstrate specific role for p42/p44 MAP kinase pathway in clonogenic activity of PC3 cells, and suggest critical role for this MAP kinase pathway in metastasis of hormone independent prostate cancer.

#4299 Interleukin-17 cytokines and novel receptor-like protein in prostate cancer. Dominik R. Haudenschield, Timothy A. Moseley, and A. Hari Reddi. *University of California at Davis Medical Center, Sacramento, CA.*

Chronic inflammation of the prostate may be a contributing factor in the development of prostate cancer. Members of the newly identified interleukin-17 cytokine family are produced by a variety of tissues including the prostate, although the founding member, IL-17, is expressed only by T-cells and B-cells. The functions of IL-17 cytokines in the prostate are not known. However, in other tissues IL-17s induce the

expression of IL-6, IL-8 and iNOS, synergistically increase the effects of pro-inflammatory cytokines including IL-1 beta, IFN-gamma and TNF-alpha, and enhance the recruitment of neutrophils and macrophages. We therefore investigate the potential role of IL-17s in prostate and prostate cancer. We document the expression of IL-17 cytokines in the prostate, and describe the cloning and characterization of a novel type I single-pass transmembrane protein with homology to the IL-17 receptor (named IL-17 Receptor-Like, IL-17RL). High mRNA levels of IL-17RL were detected in prostate, cartilage, kidney, liver, heart, and muscle by northern blot. At least 14 RNA splice variants were found, transcribed from 19 exons on human chromosome 3. Alternative splicing was predicted to introduce premature stop codons, which often occur before the transmembrane domain. Translated proteins are predicted to range from 186 to 720 amino acids in length, and can be classified as either transmembrane or secreted proteins. The transmembrane proteins have cytoplasmic tyrosines, serines and threonines and thus the potential for signal transduction. The soluble secreted proteins lack the transmembrane and intracellular domains, and may function as soluble 'decoy receptors', retaining the ligand binding domain but acting as antagonists to cytokine signaling. Differential exon usage was found in different tissues by quantitative RT-PCR, raising the possibility that alternative splicing may regulate the activity of this pathway. Using antibodies directed to the cytoplasmic and the extracellular domains of IL-17RL, we investigated its expression in human prostate biopsies. Proteins corresponding in size to the soluble and the transmembrane isoforms are present in homogenized prostate biopsy, by western blot analysis. IL-17RL is distributed in both the epithelial and the stromal components of normal prostate, shown by immunohistochemistry. Moreover, there is evidence that the expression and distribution of IL-17RL may be altered in higher grades of carcinoma, and in areas of inflammation.

#R4300 IKK1 is a component of the positive feedback loop involved in the constitutive activation of NF- κ B in prostate carcinoma cells. Alexander Yemelyanov, Ya Juan Yao, and Irina V. Budanova. *AMC Cancer Research Center, Denver, CO.*

Our recent data and data by others indicate that NF-kappaB is constitutively activated in androgen-independent prostate carcinoma (PC) cells and prostate tumors, and that NF-kappaB activation promotes PC cell tumorigenicity, invasiveness and resistance to apoptosis. The important step in NF-kappaB activation is the phosphorylation of IkappaB inhibitor proteins by IKK kinases: IKK α , IKK β and IKK-related inducible kinase IKK1. IKK1 is the only IKK whose activity is regulated by its expression. We found that IKK α and IKK β were uniformly expressed in primary prostate cells and PC cell lines. On contrast, IKK1 was strongly expressed only in androgen-independent PC cells (PC3 and DU145) with high level of constitutively active NF-kappaB but not in androgen-dependent PC cell lines (LNCaP and MDA PCa 2b) and primary prostate epithelial cells. Immunostaining also revealed that IKK1 was expressed in human prostate carcinomas. Treatment of PC cells with NF-kappaB inducers such as IL-1 and TNF-alpha resulted in a rapid induction of IKK1. Transient transfection of different PC cell lines with IKK1 w.t. resulted in activation of kB-Luciferase reporter, whereas IKK1 dominant negative (d.n.) mutant K38A suppressed basal NF-kappaB activity in PC cells. These data provide experimental evidence that IKK1 could be involved in the regulation of NF-kappaB activity in PC cells through a positive feedback loop. Supported by DOD Prostate Cancer Research Program DAMD 17-01-1-0015 and University of Colorado prostate SPORE developmental program.

#R4301 Bone morphogenetic protein signaling in prostate cancer cell lines. Kristen D. Brubaker, Eva Corey, Lisha G. Brown, and Robert L. Vessella. *University of Washington, Seattle, WA.*

Bone morphogenetic proteins (BMPs), a subfamily of the transforming growth factor- ϵ^2 (TGF- ϵ^2) superfamily, regulate many developmental processes and are involved in bone formation. BMPs signal through a complex of type I and II receptors, which initiates a cascade of events that regulates these processes. Prostate cancer cells were found to express BMPs, which might contribute to the development of the osteoblastic lesions associated with CaP. Additionally, CaP cells were found to express BMP receptors (BMPRs) suggesting a possibility of direct effects of BMP on CaP cells. In this study we evaluated the

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High sensitivity of prostate carcinoma cell lines to NF-[kappa]B induction.

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One of the central mechanisms protecting cells from apoptotic death is mediated by NF-[kappa]B transcriptional factors that control function of numerous cell survival genes. Our recent data and data by others showed that NF-[kappa]B is constitutively activated in androgen-independent prostate carcinoma (PC) cells and prostate tumors, and that NF-[kappa]B activation promotes PC cells resistance to apoptosis induced by chemo-therapeutical compounds. The results of our experiments indicated that androgen-independent PC cells maintain the high level of NF-[kappa]B basal activity by employment of the mechanism similar to that for NF-[kappa]B activation by inducers such as cytokines. This includes constitutive IKK activation, phosphorylation and fast turnover of I[kappa]B[alpha] inhibitor in androgen-independent PC cells. To find whether the high basal level of NF-[kappa]B activity in PC cells affects further NF-[kappa]B induction, we analyzed the sensitivity of normal prostate epithelial cells and PC cell lines to the standard NF-[kappa]B inducers such as TNF-[alpha], TPA and LPS. The results of our experiments showed that in contrast to other tumor cell types with constitutively activated NF-[kappa]B, PC cells independently on the basal level of NF-[kappa]B, are highly sensitive to NF-[kappa]B activation. The lack of response of LNCaP cells to LPS and DU145 cells to TPA rather reflects the cell-specific changes in the upstream signaling than function of NF-[kappa]B transcription factor. Supported by DOD Prostate Cancer Research Program DAMD17-01-1-0015.

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